Phycoerythrin Signatures In The Littoral Zone

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LONG-TERM GOALS

My long-term goal is understanding ecosystem-level impacts of the species-specific success among different phytoplankton taxa. Does it matter which species dominate? Why and when do specific taxa dominate? I am particularly interested in the way that evolutionary diversification in the architecture and pigment organization of different phytoplankton groups influences their distribution among different optical environments. To this end, I have been working with experts in remote sensing and ocean optics toward the development of an "optical biogeography" for marine picophytoplankton. I have focused on different spectral forms of marine *Synechococcus* and *Prochlorococcus* because of their distinct pigmentation, size, and apparent niche.

OBJECTIVES

The term 'phycoerythrin' (PE) refers to a family of light harvesting pigment proteins that provide the characteristic red color to many strains of marine *Synechococcus, Trichodesmium,* essentially all red algae, and several other groups of microalgae and cyanobacteria. Different spectral forms of PE harvest light in different regions of the spectrum depending on the relative concentration of two different chromophores; these can be incorporated into the assembled PE molecule in various combinations. All PEs contain the chromophore phycoerythrobilin (PEB, λ_{AbsMax} ~550 nm), which effectively harvests green light. In many forms of PE the absorption of shorter wavelengths that penetrate seawater more efficiently is enhanced by the presence of second chromophore. phycourobilin (PUB, λ_{AbsMax} ~500 nm). PE is a highly fluorescent molecule and the relative abundance of the two chromophores can be estimated from the shape of the fluorescence excitation spectrum for PE emission (Wood et al. 1985). In most marine environments, PE containing organisms are so abundant that it is also relatively simple to characterize the dominant spectral form of PE in bulk seawater using fluorescence excitation spectroscopy (Wood et al. 1998, 1999). Preliminary work in the Arabian Sea and western Atlantic indicate that there is a dependable association of different spectral forms of PE and different optical environments (Wood et al. 1999, unpublished; Sherry and Wood 2001). These observations led me to make the following prediction about the optical biogeography of different spectral forms of PE:

PEB- lacking spectral form of PE	Case II Waters
Low PUB spectral form of PE	"Green" [$K_d(440)>K_d(550)$] Case I Waters
High PUB spectral form of PE	"Blue" [$K_d(440)<K_d(550)$] Case II Waters

The primary objectives of this project are: 1) to test the hypothesis that this is a robust prediction across a wide range of marine ecosystems, 2) to determine if there is a correlation between the spectral signature of PE in different environments and the apparent and inherent optical properties of these environments and, 3) to determine if any of these parameters can be used to predict the relative importance of PE containing organisms in the plankton. A fourth objective of the ongoing work has been to understand the biological mechanisms that underlie changes in the spectral signature of PE in a water mass. Do these changes arise from physiological adaptation by the same community of organisms, or do these changes reflect changes in the genetic composition of the community (or both)?

APPROACH

My approach to the principal objectives of this project is to characterize the PE spectral signature of bulk seawater using scanning fluorescence spectroscopy, and to compare the PE spectral signature to optical properties measured in wide range of marine environments. This work is being coordinated with collaborators who are funded separately to make optical measurements: S. Pegau, R. Zaneveld, and T. Cowles (Oregon State University); R. Arnone, R. Gould, A. Weideman, and C.Davis (NRL); and C. Trees, J. Mueller, H. Maske (CHORS). I am very grateful for their willingness to contribute to this research.

We are approaching our fourth objective by examining the genetic basis of the phenotype in a range of strains grown in the laboratory, and by doing physiological experiments to screen these isolates for phenotypic plasticity in the PE spectral signature. The physiological experiments involve acclimating strains to white light conditions, altering the spectral quality of available light with filters, maintaining roughly constant levels of total photon flux density, and monitoring PE spectral signature using scanning fluorescence spectroscopy. If there has been no change in spectral phenotype after 20 generations, we consider that the strain is incapable of chromatic adaptation on ecologically meaningful timescales. We have also begun to examine the organization and copy number of the PE genes in several strains in order to see if there is a correlation between the basic spectral phenotype (PUB+ or PUB- when grown in white light) and the number of different forms of PE in the strains. Work reported by Glazer and colleagues (Ong and Glazer 1987; Wilbanks et al. 1991; Wilbanks and Glazer 1993a,b) suggest that only PUB+ strains have the potential for chromatic adaptation by differential expression of two or more forms of PE because the PUB- strain they examined had only one copy of PE. If this pattern holds true for most strains exhibiting the PUB- phenotype, it means that these strains, which we also predict to be affiliated with "Green" Case II waters, would be likely to have a genetically determined spectral phenotype. This is an important issue since physiological stability of the spectral phenotype would allow us to develop the PUB- spectral signature as a diagnostic marker for Case II waters in regions where complex circulation patterns lead to a mosaic of "green" Case I water and "green" Case II water.

WORK COMPLETED

We have participated in four cruises to distinctly different optical environments: the West Florida Shelf, the Northern Gulf of Mexico, the Gulf of California, and the New Jersey Continental Shelf. As noted above, this work has been coordinated with investigators from NRL (West Florida Shelf, Northern Gulf of Mexico, New Jersey Continental Shelf), Oregon State University (Gulf of California and the New Jersey Continental Shelf), and CHORS (Gulf of California and Northern Gulf of Mexico).

Data analysis is completed or nearly completed for all cruises and we are in the process of writing manuscripts reporting the results highlighted below (See 'Results').

A large number of strains from our collection have been screened for the ability to chromatically adapt, and this work will continue throughout the duration of the grant. At present, we have not identified any chromatic adapters among the strains currently in our collection. Our efforts to characterize the copy number and diversity of PE genes in cultured isolates depended first on our ability to prepare DNA from marine picocyanobacteria and to amplify and sequence target genes. To begin with, we built this capability in the lab by using standard protocols for sequencing ribosomal DNA since this is widely performed and the sequence of 16S rDNA provides considerable phylogenetic information (Wood et al. 2002; Wingard et al. 2002). This year, we have begun to work directly on the PE genes and have successfully amplified and sequenced the β-PE gene from one of our PUB-strains.

RESULTS

As noted above, we have developed the capability to extract and amplify DNA from marine picocyanobacteria in my lab. One of our publications demonstrates the existence of even greater phylogenetic diversity among the PE-containing picocyanobacteria than has been previously reported (Wingard et al. 2002). We have also published a molecular phylogeny demonstrating the close association of some *Synechococcus* strains with some filamentous cyanobacteria (Wood et al. 2002). We now have preliminary data on the sequence of the β subunit of the PE gene from one of our PUB-strains. We appreciate the assistance of Wolfgang Hess (Hess et al. 1996,1999) in primer development.

Our fieldwork, which now includes sampling from a wide range of Case I and Case II water masses, has shown clearly that the low PUB-PE spectral forms are associated with "green" Case I waters, specifically oceanic waters enriched by coastal upwelling. Further, the spectral signature associated with PUB-lacking PEs is restricted to highly turbid nearshore waters (e.g. in the nearshore regions of the Northern Gulf of Mexico, and inshore regions of the New Jersey continental shelf). In these regions, single cell analysis suggests that there is a diverse range of cell types, all conforming generally to the "low" PUB spectral type. High PUB-PEs consistently dominate the PE spectral signature of bulk water from "blue" Case I environments.

Our data have provided a good opportunity for examining the comparative biogeography of *Prochlorococcus* and PE-containing picocyanobacteria. *Prochlorococcus* is clearly an open ocean specialist and its presence on the shelf is an indication of the intrusion of offshore water. Additionally, while PE-containing cells are usually co-dominant with *Prochlorococcus* offshore, they actually achieve their highest abundance on the continental shelf in very turbid, shallow, water. This pattern was clearly observed in the Gulf of Mexico off west Florida and at CoJET sampling sites, but also on the New Jersey shelf where we were sampling at the LEO-15 study site and working with collaborators funded through the HYCODE initiative. Our sampling region in the HYCODE experiment extended from extremely nearshore (<20m isobath) to the 1000m isobath. As can be seen in Figure 1, there is a strong east-west axis of abundance and we see a dramatic shift in the abundance of *Prochlorococcus* and PE-containing *Synechococcus* at approximately 73 W longitude.

A surprising finding was the presence of high concentrations of phycocyanin-dominant *Synechococcus* on the mid-shelf regions of the HYCODE study area (Fig. 1). Phycocyanin is a water-soluble pigment closely related to phycoerythrin. It is a blue pigment found in nearly all cyanobacteria.

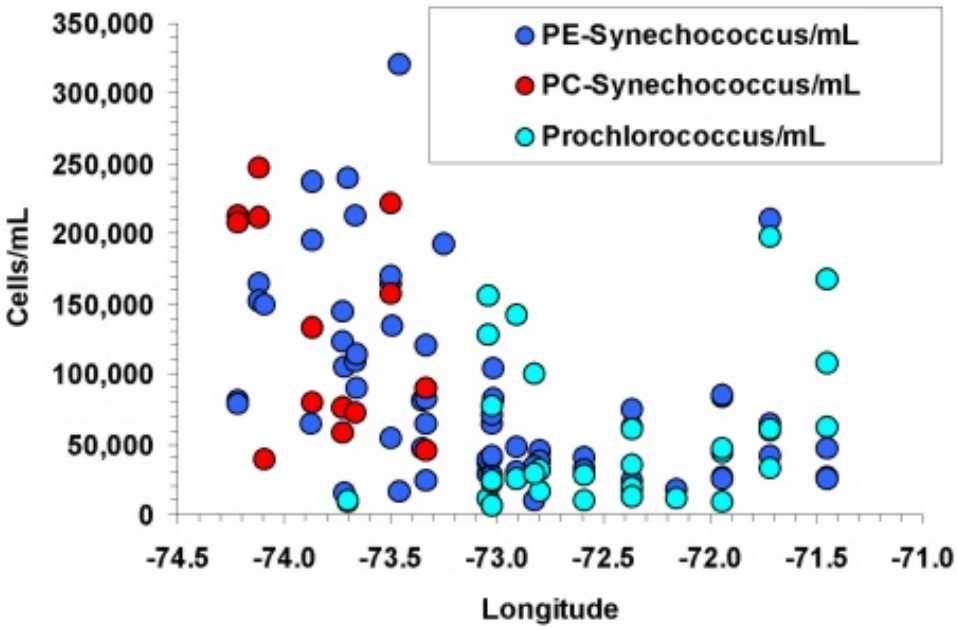

Figure 1. Scatter plot of cell abundance vs. longitude for three major classes of picocyanobacteria off the New Jersey coast during summer of 2001 (HYCODE study region). The data show a sharp transition in community structure at -73 ° (73 °W). East of -73 °, Prochlorococcus are common and reach fairly high densities (>100,000 ml^{-1}), PE-containing Synechococcus are present at 10^4 ml^{-1}, and phycocyanin (PC)-dominant Synechococcus are completely absent. West of -73, Prochlorococcus are essentially completely absent, and both PE- and PC- dominant Synechococcus achieve very high densities (>200,000 cells ml^{-1}).

However, in PE-containing S*ynechococcus* PC is present in trace amounts and its fluorescence and reflectance are masked by PE. PC-dominant picocyanobacteria are known to be at an extreme disadvantage in most oceanic waters because of their requirement for red light and are generally assumed to occur exclusively in estuarine waters and in benthic communities (Wood, 1985; Glover et al. 1987). Their presence in our samples was confirmed by flow cytometry, epifluorescence microscopy, and scanning spectrofluorometry. At some stations, these cells contributed significantly to the excitation of fluorescence emission by chlorophyll.

Data from our Gulf of California cruise has provided an outstanding data set for identifying distinctive optical properties associated with blooms of PE-containing cyanobacteria. From the combined biological and optical data we were able to compare optical properties in water masses dominated by different phytoplankton communities, including stations dominated by diatoms, by picocyanobacteria (*Prochlorococcus* and *Synechococcus*), and by *Synechococcus alone.* The results indicate that *Synechococcus* blooms can be discriminated from other phytoplankton communities by optical signals

detectable by remote sensing (Wood et al. 2002b). This is important because such blooms appear to be fairly common (Glover et al. 1988; Morel 1997; Bidigare et al. 1997) and they represent unique incidents where upwelling or storm-driven mixing support productivity that will enter the microbial loop instead of a food web that contributes to export flux.

IMPACT/APPLICATIONS

This work is providing a new picture of the niche(s) occupied by picocyanobacteria in the ocean. Rather than being primarily oceanic, the marine *Synechococcus* clearly represent a functional group with a wide range of morphological and spectral types. In general, they consistently maintain higher concentrations in warm coastal waters than in any other region of the sea. Thus, they represent a major food source to filter feeding plankton, including invertebrate larvae and benthic filter feeders. The data from this project indicate that PE spectral signature is an informative oceanographic parameter. It can provide information on the coherence of water masses, the distribution of Case II waters in complex oceanographic settings, and the occurrence of picoplankton blooms in upwelling regions where larger normally dominate.

TRANSITIONS

I would like to transition some of this work to direct application in remote sensing. I am interested in both the development of algorithms for estimating the biomass of PE-containing cyanobacteria from data on ocean color and the development of methods for characterizing the PE spectral signature from aircraft and satellites.

RELATED PROJECTS

Funding for collection of field data has been through a number of projects funded to my collaborators: SIMBIOS projects of Ron Zaneveld, Scott Pegau, Jim Mueller, and Chuck Trees. HYCODE projects of Scott Pegau, Bob Arnone, and Oscar Schofield, and the CoJET Ocean Color project of Bob Arnone and Chuck Trees. Molecular methods being used in this project were developed at an enhanced rate because the work complemented a separate study on spectral and genetic diversity in cyanobacteria from the Salton Sea funded by the EPA. Flow cytometric analysis is funded largely by the Canadian Dept. of Fisheries and Oceans through a longstanding collaboration I maintain with W.K.W. Li (Bedford Inst. Oceanography, Dartmouth, N.S., Canada).

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